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Research paper

Sensitive detection of sulfate in $PM_{2.5}$ via gold nanoparticles/poly-L-lysine/graphene composite film based arylsulfatase-inhibition biosensor

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ABSTRACT

This work developed a novel sulfate biosensor based on arylsulfatase (AS) inhibition. AS mixed with glutaraldehyde and bovine serum albumin was immobilized on glassy carbon electrode (GCE), which was first modified with poly-L-lysine (PLL)/graphene (GR) hybrid and electrodeposited gold nanoparticles (AuNPs). The characterizations were performed by TEM, SEM, FTIR, Raman spectroscopy and cyclic voltammetry. AS can catalyze 4-nitrocatechol sulfate (NCS) to hydrolyze to sulfate and 4-nitrocatcchol (NC), which is electroactive and an anodic current occurs at ~0.42 V vs. SCE. However, the production of NC would decrease in the presence of sulfate due to its inhibition effect on the enzymatic hydrolysis reaction, thus leading to a decrease in the oxidation current. The inhibition measurements were carried out by detecting this current decrease after adding sulfate. At optimal conditions, the inhibition rate was linear to −log [sulfate] in the concentration range from 1.0×10^{-7} to 1.0×10^{-5} M with the detection limit of 4.0×10^{-8} M. The introduced biosensor was further applied to assay the sulfate constituent in $PM_{2.5}$, and the results were verified by employing ion chromatography as a reference. It showed that the new biosensor has a promising application for sulfate detection in real samples.

1. Introduction

Airborne particulate matter (PM), especially the fine fraction ($PM_{2.5}$, aerodynamic diameters ≤2.5 μm), is one of the most important environmental pollution problems worldwide and recently caused increasing concern due to their adverse effects on air quality, visibility reduction, atmospheric chemistry, global climate system, and human health [1,2]. $PM_{2.5}$ usually control mass concentrations of total PM, last longer in ambient air and pose greater health risks than coarse particles because of their small particle sizes [3,4]. So, a study on $PM_{2.5}$ of their sources, components and formation mechanisms becomes more and more important, which will help to assess their detrimental impacts on environment, human health, and developing mitigation strategies. It has been reported that $PM_{2.5}$ originate either directly from biomass combustion, vehicle exhaust, industry activities and other natural and anthropogenic sources or by conversion from gaseous precursor pollutants via secondary atmospheric chemistry [5,6]. Their main constituents are water-soluble inorganic ions (such as SO_4^{2-}, NO_3^-, Cl^-, F^-, NH_4^+, K^+, Na^+, Ca^{2+} and Mg^{2+}), carbonaceous species (organic and elemental carbon), crustal elements (such as Fe, Al, Ca, Mg and K) and trace elements (such as Zn, Cu, Pb, Cr, and Cd) [7]. Water-soluble inorganic ions, that are dominated by secondary inorganic aerosols (SO_4^{2-}, NO_3^-, NH_4^+), usually account for the main fraction of the $PM_{2.5}$ mass concentration and have significant contribution to the degradation of visibility and the acidity of aerosols [8–10]. Accordingly, various instruments have been exploited for monitoring sulfate constant in $PM_{2.5}$, such as aerodyne aerosol mass spectrometer (AMS), Dionex gas particle ion chromatograph (GP-IC), particle-into liquid sampler with ion chromatograph (PILS-IC), Thermo model 5020 sulfate particle analyzer, Rupprecht & Patashnick 8400S particulate sulfate monitor, and so on [11–13]. But

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these instruments are generally large, complex, expensive, time-consuming for sample preparation, and inconvenient for in situ applications. In addition, they show serious underestimation of sulfate concentrations and often reduced by 20–50% of those determined from $PM_{2.5}$ filter samples [13]. Therefore, for environmental protection and public health safety purposes, it is of great importance to explore a new analytical method for sulfate detection in $PM_{2.5}$.

As an alternative to the traditional assay techniques, biosensors have been very popular in the environmental detection applications during the last decade [14,15]. Especially, the electrochemical biosensors, the most prevalent type and being successfully commercialized, offer a rapid and inexpensive approach for sensitive and on-site monitoring [16,17]. They have advantages over the others owing to their high instrumental sensitivity, miniaturization feasibility, operability in complex media and non-destructiveness to the testing system [17,18]. Unfortunately, in spite of the great interest in exploiting electrochemical biosensors for sulfate detection, so far few relevant reports have emerged in the literature. Nonetheless, Cserfalvi and Guilbault have developed one based on the inhibition of sulfate to the hydrolysis of arylsulfate catalyzed by arylsulfatase (AS) on the Pt disc electrode in 1976 [19]. Although the reported electrochemical biosensors based on enzyme-inhibition have appeared to be a promising technique for environmental monitoring and been widely applied in the determination of pollutants (including heavy metal ions, pesticides and other hazardous materials) [20,21]. The above inhibition based sulfate biosensor had poor detection limit and it has not been used for real sample analysis. To help address this problem, newly emerged nanomaterials and advanced electrical techniques were employed to enhance the performance of sulfate electrochemical biosensor in this study.

Utilizing various nanomaterials (such as graphene, carbon nanotubes, semiconductors, metallic oxides, and noble metal nanoparticles) to ameliorate the performances of electrochemical biosensors has attracted considerable attention due to their potential advantages over past decades [22]. Among them, gold nanoparticles (AuNPs) are very popular for their unique structure, including high specific surface area, good biological compatibility, excellent conducting capability, superior optical, catalytic, and magnetic properties [23,24]. Recently, an increasing number of electrochemical biosensors have been constructed by using AuNPs incorporated with graphene because such nanocomposite has synergistic effects on sensing applications and can improve the sensor's sensitivity [25–28]. Graphene (GR), a two-dimensional material consisting of sp^2 bonded carbon atoms compactly packed into a honeycomb structure and has shown wide-spread applications in bioelectrochemistry for its remarkable optical, thermal, mechanical, electronic and electrochemical properties, and additionally low cost and low environmental affect [29–32]. It can be used as an electrode modifying material to amplify signals or a nanocarrier to load more biomolecular recognition elements, which will further provide a biocompatible environment to immobilize biomolecules [33]. Whereas, GR sheets are apt to aggregate irreversibly or even restack into graphite through the strong van der Waals and π–π stacking interactions if they haven't been fully separated from each other, which may limit the full play of their excellent properties, especially in electrochemical biosensors [34–36]. Necessary protocols should be taken to prevent GR nanosheets from aggregation. Actually, a number of strategies have been carried out to prevent the aggregation of GR, such as covalent and non-covalent functionalization with polymers or other molecules [37]. Poly-L-lysine (PLL), as a kind of efficient polymer, exhibits enormous potential advantages in virtue of its flexible molecular backbone, good biocompatibility, and comparatively good water solubility [38]. Water-soluble GR sheets and a very biocompatible environment for further functionalization can be obtained conveniently through the functionalization of biocompatible PLL [39,40]. Furthermore, with plentiful free positively charged active $-NH_2$ in its structural framework, PLL (pKa = 10.4) can be used to detect anions [41]. More effects have been performed to apply PLL in biosensors construction [42,43].

In the present work, we developed an electrochemical biosensor for sulfate determination based on enzyme-inhibition by integrating the special characteristics of AuNPs, PLL, GR and AS from Helix pomatia. The enzyme AS cross-linked in glutaraldehyde and bovine serum albumin matrix was immobilized on the composite film of AuNPs/PLL/GR formed on a glassy carbon electrode (GCE). The obtained AS/AuNPs/PLL/GR/GCE was applied to detect sulfate based on its inhibitory action on the enzymatic hydrolysis reaction. In the reaction catalyzed by AS, the substrate 4-nitrocatechol sulfate (NCS) was hydrolyzed into 4-nitrocatcchol (NC) and sulfate. The electroactive product of NC can be electrochemical oxidized and an anodic current would be observed. But the formation of NC would be reduced in the presence of sulfate because of the competitive inhibitory effect of sulfate on the hydrolysis reaction, thus resulting in a current response decrease. Therefore, the inhibition measurements were achieved by determining this current decrease after the inhibitor (sulfate) was added. And by plotting the inhibition rate against the sulfate concentration, the calibration curve would be obtained. It was worth pointing out that the synergic effect of PLL-functionalized GR and AuNPs led to a good performance of the biosensor. Compared with our previous work [44], the present work was concentrated on the development of an enzyme inhibition biosensor for the electrochemical detection of sulfate and it has displayed a better analytical performance. The proposed biosensor was further applied to assay the sulfate constituent in $PM_{2.5}$ of Yangzhou in China. And the results obtained by this novel electrochemical biosensor were consistent with those obtained by the classical ion chromatography (IC) method.

2. Experiments

2.1. Reagents and materials

Arylsulfatase isolated from Helix pomatia (AS, Type H–1 ≥ 10,000 units/g solid), bovine serum albumin (BSA, ≥98%), and 4-nitrocatechol (NC, >97%) were purchased from Sigma-Aldrich (USA). 4-Nitrocatechol sulfate (Dipotassium salt hydrate, NCS, >95%) was obtained from Tokyo Chemical Industry Co., Ltd. (Japan). Chloroauric acid tetrahydrate (≥47.8% as Au) and glutaraldehyde (GA, 25%) was provided by Sinopharm Chemical Reagent Co., Ltd. (China). Poly-L-lysine (PLL, Mw = 30000-70000, 10% w/V) was provided by Chengdu Xiya Chemical Co., Ltd. (China). Graphene (GR) was a product of Nanjing Ji Cang Nano Technology Co., Ltd. (China). $PM_{2.5}$ samples were obtained from the Yangzhou Municipal Environmental Protection Agency. Sulfate standard substance (1000 mg/L) was procured from National Institute of Measurement and Testing Technology (China). Acetate buffer solutions with various pH were prepared by mixing 0.1 M stock solutions of acetic acid and sodium acetate. All other reagents were of analytical grade and used as received. Ultrapure water (>18 MΩ cm) was employed for preparation of aqueous solutions throughout this work.

2.2. Apparatus

Electrochemical experiments were performed at room temperature (about 25 °C) using a CHI615C electrochemical analyzer (CH Instruments, Shanghai, China) connected to a personal computer. A three-electrode system was used in the measurements, with a bared or modified glassy carbon electrode (GCE, Φ = 3 mm) as work-